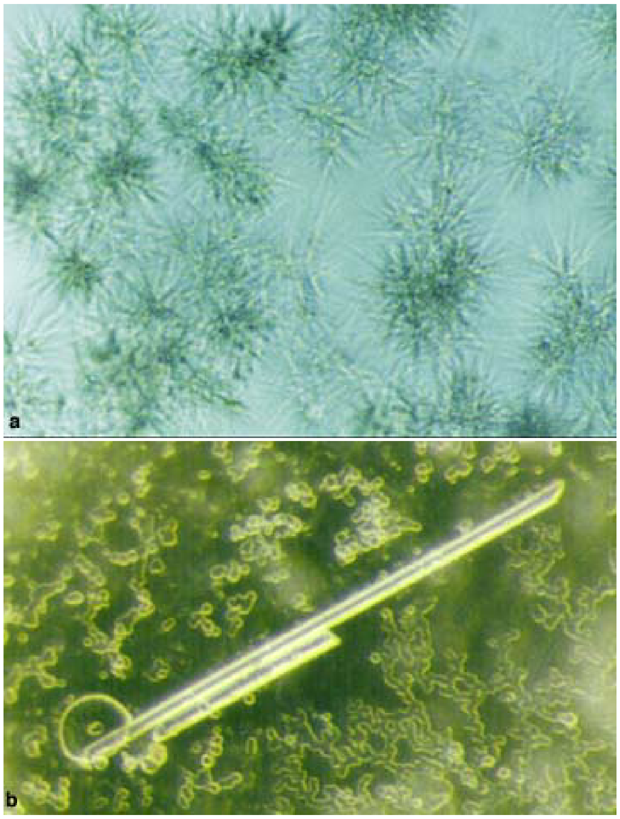
Microgravity Biotechnology Discipline Brochure



These images of crystals of the protein raf kinase, which is important to cancer research, compare results of ground-based crystal growth (**a**) to crystal growth in microgravity (**b**). The long, thin crystals in the right photo are approximately an order of magnitude larger than the small, needle-like crystals in the left photo. The space-grown crystals, which were grown during the second United States Microgravity Laboratory (USML-2) mission in November 1995, were the largest crystals of raf kinase ever produced. Large, uniform crystals like these generally yield better structural information when analyzed through X-ray diffraction, which in turn can lead to a better understanding of how the structure of a protein is related to its function in the human body.

What Is Biotechnology?

Biotechnology is an applied biological science that involves the research, manipulation, and manufacturing of biological molecules, tissues, and living organisms. With a critical and expanding role in health, agriculture, and environmental protection, biotechnology is expected to have a significant impact on our economy and our lives in the next century. Focused on three principal areas of research -- protein crystal growth, mammalian cell and tissue culture, and fundamental biotechnology -- the microgravity biotechnology program has benefited from using low-gravity environments to grow protein crystals, cells, and tissues in experiments completed thus far. Marshall Space Flight Center in Huntsville, Alabama, is NASA's Microgravity Center of Excellence for biotechnology and is supported by the biotechnology program office at Johnson Space Center in Houston, Texas.

Why Conduct Biotechnology Research in Microgravity?

Gravity significantly influences attempts to grow protein crystals on Earth. Research on the space shuttle and the Russian space station, *Mir* , has indicated that protein crystals grown in microgravity can yield substantially better structural information than can be obtained from crystals grown under the full influence of Earth's gravity. Proteins consist of thousands -- or in the case of viruses, millions -- of atoms, which are weakly bound together, forming large molecules. On Earth, gravity-driven phenomena such as buoyancy-induced convection (flows caused by temperature-driven density differences in a fluid) and sedimentation (the separation of materials of different densities) may inhibit crystal growth. In microgravity, convection and sedimentation are significantly reduced, allowing for the production of better and larger crystals.

The absence of sedimentation means that protein crystals do not sink to the bottom of their growth container as they do on Earth. Consequently, they are not as likely to be affected by other crystals growing in the solution. Since convective flows are also greatly reduced in microgravity, crystals grow in a much more stable environment, which may be responsible for the improved structural order of space-grown crystals. Knowledge gained from studying the process of protein crystal growth in microgravity conditions will have implications for protein crystal growth experiments on Earth.

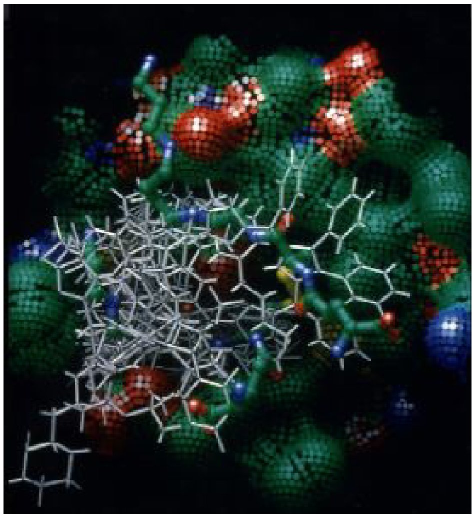
Research also shows that mammalian cell tissues -- particularly those composed of normal cells -- are sensitive to conditions found in ground-based facilities used to culture (grow) them. Fluid flows and sedimentation caused by gravity can separate the cells from each other, severely limiting the number of cells that will aggregate (come together). But tissue samples grown under microgravity conditions can be larger and more representative of tissues that are actually produced inside the human body. This suggests that better control of the stresses exerted on cells and tissues can play an important role in their culture. These stresses are greatly reduced in microgravity. (See back page for more information about microgravity, or µg.)

Biotechnology Research Areas

Protein Crystal Growth

The human body contains over 100,000 different proteins. These proteins play important roles in the everyday functions of the body, such as the transport of oxygen and chemicals in the blood, the formation of the major components of muscle and skin, and the fighting of disease. Researchers in the area of protein crystal growth seek to determine the structures of these proteins, to understand how a protein's structure affects its function, and ultimately to design drugs that intercede in protein activities (penicillin is a well-known example of a drug that works by blocking a protein's function). Determining protein structure is the key to the design and development of effective drugs.

Defining Protein Structure



Scientists study the structure of protein crystals to determine how structure affects the function of individual proteins. To conduct this type of study, scientists must first generate crystals that are large enough and uniform enough to provide useful structural information upon analysis. Protein crystals grown in microgravity are often significantly larger and of better quality than those grown on Earth. Once a high-quality crystal has been selected, it is examined through a process called X-ray diffraction, in which X-rays are directed into the crystal and are scattered in a regular manner by the atoms in the crystal. The scattered X-rays are recorded on photographic film or electron counters. This data is then fed into a computer, which can perform precise measurements of the intensity of the X-rays scattered by each crystal, helping scientists to map the probable positions of the atoms within each protein molecule.

Pictured above is the computer-generated model of the protein crystal trypanathione reductase, a protein that is essential to the vitality of a parasite that causes Chagas' disease, a devastating illness affecting the heart and gastrointestinal tract. Researchers can use their knowledge of the structure of such a protein to design drugs that will interact with the protein and inhibit its functions, thus preventing or curing a disease. Structure-based drug design techniques are being used in the search for treatments for diseases like Chagas', AIDS, diabetes, and cancer.

The main purpose in growing protein crystals is to advance our knowledge of biological molecular structures. Researchers can use the microgravity environment to help overcome a significant stumbling block in the determination of molecular structures: the difficulty of growing crystals suitable for analysis. Scientists use X-ray analysis to determine the three-dimensional structure of a protein. From high-resolution data, scientists can describe a protein's structure on a molecular scale and determine the parts of the protein that are important to its functions. Using computer analysis, scientists can create three-dimensional models of the protein and manipulate them to examine the intricacies of the protein's structure. They can then create a drug that "fits" into a protein's active site, like inserting a key into a lock, to disable the protein's function. But X-ray analysis requires large, homogeneous crystals (about the size of a grain of table salt), and unfortunately, crystals grown in the gravity environment of Earth often have internal defects that make such analysis difficult or impossible. Space shuttle missions have shown that crystals of some proteins (and other complex biological molecules, like viruses) that are grown in space, away from gravity's distortions, are larger and have fewer defects than those grown on Earth. The improved data from the analysis of space-grown crystals significantly enhance scientists' understanding of the proteins' structures, and this information can be used to support structure-based drug design.

Scientists also strive for a better understanding of the fundamental mechanisms by which proteins form crystals. A central goal of NASA's protein crystal growth program is to determine the basic science that controls how proteins interact and order themselves during the process of crystallization. The strategy for determining this is to use the knowledge gained from ground-based research and flight experiments to understand how microgravity improves the quality of protein crystals. To accomplish this goal, NASA has brought together scientists from the protein crystallography community, traditional crystal growers, and other physical scientists to form a multidisciplinary team. These collaborations are already contributing to improvements in crystal growth techniques and technology.

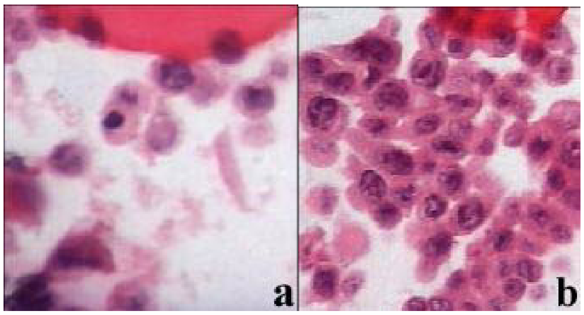
Mammalian Cell and Tissue Culture

Mammalian cell and tissue culturing is a major area of research for the biotechnology community. Tissue culturing is one of the basic tools of medical research and is key to developing future medical technologies such as *ex-vivo* (outside of the body) therapy design and tissue transplantation. To date, medical science has been unable to fully culture human tissue to the mature states of differentiation found in the body.

The study of normal and cancerous mammalian tissue growth holds enormous promise for applications in medicine. However, conventional static tissue culture methods form flat sheets of growing cells that differ in appearance and function from their three-dimensional counterparts growing in a living body. In addition, it is sometimes difficult in a static environment for growing tissue to find the fresh media (food supply) it needs to survive.

In an effort to enhance three-dimensional tissue formation, NASA scientists have developed a technology for cell and tissue culture called a rotating wall bioreactor. This instrument cultures cells in a slowly rotating horizontal cylinder, which produces lower stress levels on the growing cells than previous experimental environments designed to facilitate tissue growth on Earth. The continuous rotation of the cylinder allows the tissue sample to be suspended in growth fluid and escape much of the influence of gravity.

Another reason mammalian cells are sensitive to growth conditions found in standard stirred bioreactors is that fluid flow causes shear forces (forces that cause contiguous parts of a structure or solution to slide relative to each other) that discourage cell aggregation. This limits both the development of the tissue and the degree to which it possesses structures and functions similar to those found in the human body. The NASA bioreactor allows for a reduction of the damaging shear forces. Tissue cultures of the size that can be grown in this improved bioreactor allow tests of new treatments on patient cell cultures rather than on patients themselves. Scientists at Johnson Space Center are modifying the bioreactor, making it possible to automatically monitor and control levels of glucose, oxygen, pH, and carbon dioxide in the solution containing the tissue. In the future, this technology will enable quicker, more thorough testing of larger numbers of drugs and treatments. Ultimately, the bioreactor is expected to produce even better results in the microgravity environment achieved in orbit.



These photos compare cultures of colon carcinoma grown on the ground (a) and in the NASA bioreactor during its first spaceflight in 1995 (b). The cells in the sample grown in microgravity have aggregated to form masses that are larger and more similar to tissue found in vivo than those in the ground control sample. The space grown cells also appear to be healthier than the cells grown on Earth.

In cooperation with the medical community, the bioreactor design is being used to prepare better models of human colon, prostate, breast, and ovarian tumors. Cells grown in conventional culture systems may not differentiate to form a tumor typical of cancer. In the bioreactor, however, these tumors grow into specimens that resemble the original tumor. Similar results have been observed with normal human tissues as well. Cartilage, bone marrow, heart muscle, skeletal muscle, pancreatic islet cells, liver cells, and kidney cells are examples of the normal tissues currently being grown in rotating bioreactors by investigators. In addition, laboratory models of heart and kidney diseases and viral infections (including those from the Norwalk virus, a major cause of epidemic gastroenteritis, and the human immunodeficiency virus, or HIV) are currently being developed for further study using this technology. Continued and expanded use of the bioreactor can improve our knowledge of normal and cancerous tissue development. NASA has also started to culture tissues in the bioreactor on the space shuttle and on the Russian space station, *Mir* , where even greater reduction in stresses on growing tissue samples may allow larger tissue masses to develop. Bioreactor designs for use on the International Space Station are under way.

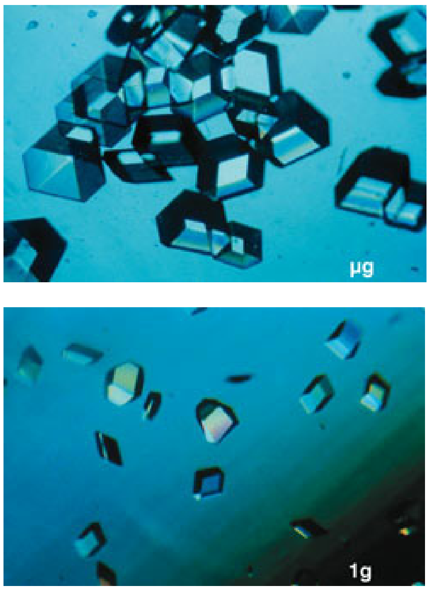
Fundamental Biotechnology

The primary purpose of research in fundamental biotechnology is to identify and understand biotechnological processes and biophysical phenomena that can be studied advantageously in a microgravity environment. Molecular and cellular aggregation, the behavior of electrically driven flows, transport processes, and capillary and surface phenomena are areas of fundamental research in biotechnology that can be applied to biological systems. Separation science and technology, particularly electrophoresis and phase partitioning, have a long history of experimentation in microgravity. Electrophoresis, which is the separation of a substance based on the electrical charge of the molecule, has been studied on a dozen space shuttle flights and has led to additional research in fluid physics.

National Aeronautics and Space Administration

Discipline Lithograph Series

MICROGRAVITY BIOTECHNOLOGY RESEARCH



Crystals of recombinant human insulin grown on Earth (1 g) are much smaller than crystals of the protein grown in orbit (µg). Insulin is a protein that enables our bodies to turn sugar, starches, and other food into energy. Understanding insulin's molecular structure may lead to better treatments or even a cure for diabetes.

WHY STUDY BIOTECHNOLOGY IN MICROGRAVITY?

Biotechnology is an applied biological science. This means that scientists use their general knowledge of biology (the study of living organisms and life processes) to solve specific problems. Two problems that scientists in NASA's microgravity biotechnology program have been working to solve are how to grow quality crystals of proteins and viruses and how to grow large three-dimensional samples of cells and tissues. Accomplishing each of these goals is a first step to important advances in medicine.

The primary obstacle to growing large crystals and tissue samples on Earth has been gravity. Both crystals and cells are typically grown in a liquid mixture. Gravity creates flows in the mixture due to density differences in the liquid (buoyancy-induced convection). It also causes heavier particles to settle to the bottom of the container (sedimentation). Because fluid flows and sedimentation disturb the growth of crystals and cells, one answer has been to conduct these growth processes in a microgravity environment, where such disturbances are virtually eliminated.

RESEARCH AREAS

PROTEIN CRYSTAL GROWTH: Proteins carry out most of the important functions in plants and animals. The human body contains over 100,000 different proteins that enable us to process nutrients, eliminate wastes, grow, reproduce, and fight disease. The function a protein performs is closely related to its molecular structure (arrangement of atoms). The ability to control the functions of proteins can lead to cures for devastating diseases such as AIDS or cancer. If the structure of a complicated protein molecule can be determined, scientists then have a key for designing a pharmaceutical drug that can "fit" the structure and influence the protein's function. Viruses, which can be crystallized in a way similar to protein molecules, often cause illness and disease in humans, animals, and plants. As with proteins, solving the molecular structure of viruses can give scientists clues for designing effective drugs to combat disease.

To get the best view of the structure of protein and virus molecules, scientists often grow crystals of them. The bigger and more orderly a crystal is, the easier it is to determine its arrangement of atoms using a technique called X-ray diffraction. Scientists direct X-rays into the crystal, where they are scattered by the atoms. The scattered X-rays are recorded on photographic film and analyzed by computers to get information to map the molecule's structure.

The size and orderliness of protein and virus crystals are limited on Earth by gravity-driven convection and sedimentation. In the microgravity environment of the space shuttle and Russian Space Station *Mir* , exceptionally high-quality crystals have been grown of many proteins and viruses, including insulin (the protein that processes sugar in the body) and turnip yellow mosaic virus (which can ruin crops).

MAMMALIAN CELL AND TISSUE GROWTH: Cells are the fundamental units of living things. Cells that group together to perform a particular function in the body, like skin, bone, or muscle, are called tissue. Inside the body (*in vivo*), cells grow and form three-dimensional structures. Researchers culture (grow) cells outside of the body in their laboratories to learn about the growth process and to have samples on which to test medical treatments. These cells are grown in media, which is a liquid containing all the nutrients that the cells need. Gravity-driven convection and sedimentation prevent these cells from forming three-dimensional structures in the same way that they do in the body.

To avoid this problem, NASA scientists have modified a bioreactor, which is a common device for culturing cells. In NASA's rotating wall bioreactor, the liquid media and the cells rotate with the walls of the container. This action suspends the cells in the media so that the effects of gravity-driven convection and sedimentation are significantly reduced. NASA has flown a rotating wall bioreactor on the space shuttle and on *Mir* to discover whether using the device in microgravity will further improve three-dimensional growth. Experiments to grow colon cancer cells on the shuttle and cartilage tissue on *Mir* have resulted in samples that look more like tissue found in the body.

WHAT IS MICROGRAVITY?

Astronauts floating in the shuttle appear weightless not because they have escaped Earth's gravity but because they are in a state of freefall. Any object falling due only to the force of gravity (freefalling) is experiencing microgravity and appears to be weightless. For example, a scale taped to an apple falling from a tree would register zero because it is falling with the apple. A spacecraft in a circular orbit is actually in a continuous state of freefall at an altitude and speed that cause its fall to match the curvature of the Earth. All objects carried by an orbiting spacecraft are also in a state of freefall and so appear to be weightless.

To learn more, try these World Wide Web addresses:

http://microgravity.msfc.nasa.gov/MICROGRAVITY/Biot.html

http://shuttle.nasa.gov/sts-81/orbit/payloads/science/bts.htm

http://microgravity.msad.hq.nasa.gov